




Association between *ALDH2* and *ADH1B* polymorphisms, alcohol drinking and gastric cancer: a replication and mediation analysis

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Abstract

Background Aldehyde dehydrogenase 2 (*ALDH2*; rs671, Glu504Lys) and alcohol dehydrogenase 1B (*ADH1B*; rs1229984, His47Arg) polymorphisms have a strong impact on carcinogenic acetaldehyde accumulation after alcohol drinking. To date, however, evidence for a significant *ALDH2*–alcohol drinking interaction and a mediation effect of *ALDH2/ADH1B* through alcohol drinking on gastric cancer have remained unclear. We conducted two case–control studies to validate the interaction and to estimate the mediation effect on gastric cancer.

Methods We calculated odds ratios (OR) and 95% confidence intervals (CI) for *ALDH2/ADH1B* genotypes and alcohol drinking using conditional logistic regression models after adjustment for potential confounding in the HERPACC-2 (697 cases and 1372 controls) and HERPACC-3 studies (678 cases and 678 controls). We also conducted a mediation analysis of the combination of the two studies to assess whether the effects of these polymorphisms operated through alcohol drinking or through other pathways.

Results *ALDH2* Lys alleles had a higher risk with increased alcohol consumption compared with *ALDH2* Glu/Glu (OR for heavy drinking, 3.57; 95% CI 2.04–6.27; *P* for trend = 0.007), indicating a significant *ALDH2*–alcohol drinking interaction ($P_{\text{interaction}} = 0.024$). The mediation analysis indicated a significant positive direct effect (OR 1.67; 95% CI 1.38–2.03) and a protective indirect effect (OR 0.84; 95% CI 0.76–0.92) of the *ALDH2* Lys alleles with the *ALDH2*–alcohol drinking interaction. No significant association of *ADH1B* with gastric cancer was observed.

Conclusion The observed *ALDH2*–alcohol drinking interaction and the direct effect of *ALDH2* Lys alleles may suggest the involvement of acetaldehyde in the development of gastric cancer.

Keywords Alcohol drinking · *ALDH2* · *ADH1B* · Gastric cancer · Interaction · Mediation analysis

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Introduction

The incidence of gastric cancer is highest in East Asia populations, at 35.4 per 100000 [1]. A major reason for this high incidence is considered to be the high prevalence of *Helicobacter pylori* (*H. pylori*) infection in this region [2, 3].

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However, the prevalence of *H. pylori* infection is not completely proportionate with the incidence of gastric cancer [4], implying the presence of other pathways in the development of gastric cancer specific to East Asian populations.

Alcohol drinking, assessed by the World Cancer Research Fund International as a probable risk factor for gastric cancer [5], may partly contribute to this incidence, given that genetic polymorphisms encoding alcohol-metabolizing enzymes vary across countries. This hypothesis may be supported by the possibly higher risk of alcohol-associated gastric cancer in East Asia than Europe and North America, as indicated in a recent pooled analysis [6]. Alcohol dehydrogenase 1B (*ADH1B*) and aldehyde dehydrogenase 2 (*ALDH2*) are key enzymes for alcohol metabolism. Alcohol is metabolized to acetaldehyde by *ADH1B* and sequentially converted to acetate by *ALDH2*. The enzymatic activity of *ADH1B* and *ALDH2* is subjected to regulation by genetic polymorphisms, and known to affect the accumulation of acetaldehyde after alcohol consumption. Among polymorphisms, *ADH1B* (rs1229984, His47Arg) and *ALDH2* (rs671, Glu504Lys) have been functionally proven to impact alcohol metabolism. *ADH1B* Arg allele carriers, which are less prevalent in Japan than in Western countries, metabolize alcohol to acetaldehyde more slowly than *ADH1B* His/His carriers [7]. *ALDH2* Lys allele carriers, which are specific to East Asian populations, have a significantly reduced capability for acetaldehyde metabolism [8, 9], and *ALDH2* Lys allele carriers tend to consume less alcohol than *ALDH2* Glu/Glu carriers owing to such acetaldehyde-related adverse effects as flushing, palpitation, nausea and headache [10]. Many epidemiological studies have shown that these polymorphisms contribute to higher susceptibility to alcohol-related cancers [11–15]. Specifically, the increased risk of esophageal cancer and head and neck cancer by the synergistic interaction between *ALDH2* Lys alleles and alcohol consumption has been reported [13]. As a result, acetaldehyde associated with alcoholic beverages has been assessed as a group 1 carcinogen by the International Agency for Research on Cancer [16].

Several case–control studies, including our previous study and a nested case–control study have investigated the association between *ALDH2* polymorphism (rs671) and gastric cancer risk in East Asian countries [17–23]. However, evidence for an interaction between *ALDH2* and alcohol drinking on gastric cancer risk is scarce. In addition, few studies have examined the association between *ADH1B* (rs1229984) and gastric cancer risk [18, 21, 22]. Accumulating evidence for an association between *ALDH2/ADH1B* polymorphisms and gastric cancer risk may help identify groups at high risk of alcohol-associated gastric cancer. Moreover, while these previous studies conducted conventional multivariate analyses with consideration to alcohol drinking as a confounder, they did not examine mediation effects between

ALDH2/ADH1B polymorphisms and alcohol drinking on gastric cancer. Given the strong impact of *ALDH2* and *ADH1B* polymorphisms (especially *ALDH2*) on alcohol drinking behaviors in East Asians [10, 24], assessment of the mediation effects of these polymorphisms through alcohol drinking may allow for focus on the mechanism of development of alcohol-related gastric cancer.

This study aimed to (1) conduct a case–control study to replicate our previous findings of the significant association and interaction between *ALDH2* polymorphism, alcohol drinking and gastric cancer risk, and (2) estimate direct and indirect effects of *ALDH2* and *ADH1B* polymorphisms on gastric cancer risk by conducting a mediation analysis from the combined data of our previous and the present case–control studies.

Methods

Participants

Participants were recruited between January 2001 and December 2005 from the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC)-2 and between December 2005 and March 2013 from HERPACC-3. The frameworks of HERPACC-2 and HERPACC-3 have been described elsewhere [25, 26]. Briefly, in each study, cases diagnosed with new-onset gastric cancer by endoscopic biopsy and controls confirmed to have no cancer and no history of neoplasm were collected from first-visit outpatients at Aichi Cancer Center Hospital in Japan. Controls were randomly matched for age (± 5 years) and sex with a case–control ratio of 1:1 or 2. As a result, the present analysis included 697 cases and 1372 controls in HERPACC-2 and 678 cases and 678 controls in HERPACC-3 (in total, 1375 cases and 2050 controls). Written informed consent was obtained from all participants. The study was approved by the institutional ethics committee of Aichi Cancer Center.

Assessment of alcohol drinking, smoking and vegetable/fruit consumption

Participants were asked to fill in a questionnaire on lifestyle information and family history of cancer before their first medical examination. The questionnaire included lifestyle information before the development of the symptoms for which they first visited the hospital. Response rate for enrollment was 97% for participants in the derivation study (HERPACC-2), of whom half provided blood samples. In the validation study (HERPACC-3), 66.4% of participants responded to the questionnaire, of whom 62% provided blood samples.

Each categorization of alcohol drinking, smoking and vegetable/fruit consumption was in accordance with that of our previously reported study (HERPACC-2) [20]. In brief, alcohol drinking status was classified as follows: (1) never drinking; (2) light drinking, defined as alcohol consumption on 4 days or fewer per week; (3) moderate drinking, defined as alcohol consumption on 5 or more days per week of less than 46 g of ethanol on each occasion; and (4) heavy drinking, defined as alcohol consumption on 5 or more days per week of 46 g or more of ethanol on each occasion. Smokers were classified as follows: (1) never smokers, defined as those who smoked fewer than 100 cigarettes in their life time; (2) former smokers, defined as those who had quit smoking for more than 1 year; and (3) current smokers. Pack-years (PY)—a measure of cumulative smoking exposure—was calculated by multiplying the number of packs of cigarettes smoked per day by the number of years of smoking. Fruit/vegetable consumption was estimated using a validated food frequency questionnaire [27]. Participants were classified into three equally sized groups according to the distribution of fruit/vegetable consumption among controls (tertiles).

Assessment of *H. pylori* infection and atrophic gastritis

H. pylori infection status and atrophic gastritis are established risk factors for gastric cancer and were accordingly considered in the evaluation [28, 29]. Plasma IgG antibody levels for *H. pylori* were measured using a commercially available direct enzyme-linked immunosorbent assay kit ('E Plate "Eiken" *H. pylori* Antibody'; Eiken Kagaku, Tokyo, Japan), with a positive infection status defined as *H. pylori* IgG > 10 U/ml in serum [20]. Serum pepsinogens (PGs) were examined by chemiluminescence enzyme immunoassay, with positive gastric mucosal atrophy defined as PG I \leq 70 ng/ml and PG I/PG II \leq 3 [20].

Genotyping of *ALDH2* and *ADH1B* polymorphisms

DNA of each subject was extracted from the buffy coat fraction with a QIAamp DNA Blood Mini Kit (Qiagen). Genotyping of *ALDH2* (rs671) and *ADH1B* (rs1229984) was conducted using TaqMan Assays with the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). We tested these two polymorphisms only because they have been shown to be associated with alcohol-related cancer, as mentioned above. The quality of genotyping was routinely assessed using the Hardy–Weinberg test.

Statistical analyses

Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using conditional logistic regression models. Unconditional logistic regression models were applied only for an analysis stratified by *H. pylori* infection status because a great number of matched controls were excluded from the analysis by conditional logistic regression models. Crude ORs (age- and sex-matched ORs) or multivariate-adjusted ORs for the association of each covariate with gastric cancer risk were estimated in HERPACC-2 and HERPACC-3 separately to allow the results of the two studies to be compared. In addition, we estimated ORs in the two studies combined to obtain more accurate point estimates. Age was categorized as < 40, 40–49, 50–59, 60–69, 70–79 years, and PY as 0, < 20, < 40, < 60, \geq 60. Multivariate analyses adjusted for age (continuous), alcohol drinking status (category: never, light, moderate, heavy), PY of smoking (category: 0, < 20, < 40, < 60, \geq 60), fruit/vegetable intake (category: lowest tertile, middle tertile, highest tertile), family history (yes, no), *H. pylori* infection (positive, negative) and gastric atrophy (positive, negative). Linear trends (*P* for trend) were tested by assigning ordinal variables in drinking categories as continuous variables in each model (never = 0, light = 1, moderate = 2, heavy = 3). Interaction terms between alcohol drinking status and *ALDH2/ADH1B* polymorphisms were included in the conditional logistic regression models to examine whether the association of alcohol consumption with gastric cancer was modified by these polymorphisms.

A mediation analysis was conducted to further assess whether the effects of *ALDH2/ADH1B* polymorphisms on gastric cancer risk operated through alcohol drinking behaviors or through other pathways. This analysis could decompose the total effect into direct and indirect effects [30]. The direct effect was the effect of these polymorphisms on gastric cancer risk through pathways other than change in alcohol drinking behaviors, while the indirect effect was the effect mediated through change in alcohol drinking behaviors. We used methods which can take an exposure–mediator interaction into consideration (paramed command in STATA) [31], given that such gene–environmental interaction between *ALDH2* and alcohol drinking on gastric cancer risk was observed in several previous studies [19, 20]. The paramed command performs causal mediation analysis using parametric regression models as an extension of Baron and Kenny method [32], which is known as a causal steps approach [33]. We compared two models: a model for the mediator (alcohol consumption) conditional on the exposure (*ALDH2/ADH1B* genotypes) and covariates, and a model for the outcome conditional on the exposure, the mediator and covariates. In the comparison, a counterfactual framework allowing for an interaction between the *ALDH2/ADH1B* genotypes and alcohol consumption was used to estimate

direct and indirect effects [34]. Missing values were treated as dummy variables in the models of the multivariate analysis and excluded in the mediation analysis.

All statistical analyses were performed using STATA statistical software version 13.1 (Stata Corp LP, College Station, TX, USA). Two-sided *P* values <0.05 were considered to show statistical significance.

Results

Table 1 shows the characteristics of case and control participants in HERPACC-2 and HERPACC-3 separately. Sex was well-balanced due to matching in both of the studies, although age was significantly higher among cases than controls only in HERPACC-2. Current smokers and

Table 1 Characteristics of cases and controls in HERPACC-2 and HERPACC-3

	Derivation study (HERPACC-2)			Validation study (HERPACC-3)		
	Case (n=697)	Control (n=1372)	<i>P</i> value ^a	Case (n=678)	Control (n=678)	<i>P</i> value ^a
Age			<0.001			0.997
<40	34 (4.9)	146 (10.6)		24 (3.5)	25 (3.7)	
40 to <50	72 (10.3)	154 (11.2)		50 (7.4)	52 (7.7)	
50 to <60	245 (35.2)	429 (31.3)		177 (26.1)	173 (25.5)	
60 to <70	210 (30.1)	435 (31.7)		283 (41.7)	287 (42.3)	
70–	136 (19.5)	208 (15.2)		144 (21.2)	141 (20.8)	
Mean age ± SD	59.4 ± 10.5	56.8 ± 12.7	<0.001	61.4 ± 9.73	61.4 ± 9.70	0.976
Sex			0.930			1.000
Male	521 (74.8)	1028 (74.9)		503 (74.2)	503 (74.2)	
Female	176 (25.3)	344 (25.1)		175 (25.8)	175 (25.8)	
Smoking status			<0.001			0.002
Never	222 (31.9)	538 (39.2)		228 (33.6)	290 (42.8)	
Former	181 (26.0)	403 (29.4)		235 (34.7)	222 (32.7)	
Current	294 (42.2)	430 (31.4)		212 (31.3)	165 (24.3)	
Unknown	0	1 (0.1)		3 (0.4)	1 (0.2)	
PY			<0.001			0.003
0	222 (31.9)	539 (39.3)		229 (33.8)	290 (42.8)	
<20	99 (14.2)	286 (20.9)		99 (14.6)	107 (15.8)	
<40	160 (23.0)	272 (19.8)		142 (20.9)	119 (17.6)	
<60	117 (16.8)	153 (11.2)		117 (17.3)	83 (12.2)	
≥60	92 (13.2)	113 (8.2)		70 (10.3)	54 (8.0)	
Unknown	7 (1.0)	9 (0.7)		21 (3.1)	25 (3.7)	
Fruit/vegetable intake			0.132			0.026
Lowest tertile	263 (37.7)	446 (32.5)		257 (37.9)	212 (31.3)	
Middle tertile	208 (29.8)	445 (32.4)		187 (27.6)	212 (31.3)	
Highest tertile	209 (30.0)	445 (32.4)		181 (26.7)	211 (31.1)	
Unknown	17 (2.4)	36 (2.6)		53 (7.8)	43 (6.3)	
Family history			0.013			0.038
No	544 (78.1)	1133 (82.6)		600 (88.5)	574 (84.7)	
Yes	153 (22.0)	239 (17.4)		78 (11.5)	104 (15.3)	
<i>H. pylori</i> infection			<0.001			<0.001
Negative	124 (17.8)	628 (45.8)		183 (27.0)	350 (51.6)	
Positive	573 (82.2)	744 (54.2)		495 (73.0)	328 (48.4)	
AG defined by PG testing			<0.001			<0.001
Negative	262 (37.6)	893 (65.1)		372 (54.9)	529 (78.0)	
Positive	434 (62.3)	479 (34.9)		300 (44.3)	149 (22.0)	
Unknown	1 (0.1)	0		6 (0.9)	0	

SD standard deviation, PY pack-year, AG atrophic gastritis, PG pepsinogen

^aDifferences between cases and controls were analyzed using the unpaired *t* test and Chi-squared test

heavy smokers were more frequently distributed among cases. Cases had a higher prevalence of *H. pylori* infection and atrophic gastritis than controls. As shown in Online Resource 1, smoking status, PY of smoking, *H. pylori* infection and atrophic gastritis were positively associated with gastric cancer, whereas fruit/vegetable intake was inversely associated with gastric cancer in both HEPACC-2 and HEPACC-3.

When adjusted for all covariates, no significant association between heavy drinking and gastric cancer was observed in the combined HEPACC-2 and HEPACC-3 analysis (Table 2). The multivariate-adjusted ORs of *ALDH2* Glu/Lys, Lys/Lys and Lys alleles (Glu/Lys + Lys/Lys) were 1.46 (95% CI 1.22–1.74), 1.51 (95% CI 1.09–2.10) and 1.47 (95% CI 1.23–1.75) compared with *ALDH2* Glu/Glu, respectively. *ADH1B* polymorphism was not associated with gastric cancer risk. Furthermore, *ADH1B* polymorphism did not interact with *ALDH2* polymorphism in gastric cancer risk. As shown in Online Resource 2, the significant impact of *ALDH2*

polymorphism in HEPACC-3 was compatible with that in HEPACC-2, as well as the above overall analysis.

Table 3 shows the association between alcohol drinking and gastric cancer risk according to *ALDH2* and *ADH1B* genotypes. *ALDH2* Glu/Glu carriers had no increased risk of gastric cancer even if they drank heavily, whereas *ALDH2* Lys allele carriers had a higher risk with increased alcohol consumption, with an OR of 3.57 (95% CI 2.04–6.27) for heavy drinking (P for trend=0.007). A significant interaction between alcohol drinking and *ALDH2* polymorphism on gastric cancer risk was also found ($P_{\text{interaction}}=0.024$). We also examined the association between amount of weekly alcohol consumption and gastric cancer risk, suggesting that alcohol consumption of less than 150 g/week did not increase the risk of alcohol-related gastric cancer among *ALDH2* Lys allele carriers (Online Resource 3). In contrast, *ADH1B* polymorphism did not significantly modify the association between alcohol drinking and gastric cancer risk ($P_{\text{interaction}}=0.173$). The result was consistent regardless of *H. pylori* infection status. In addition, the increased risk

Table 2 Impact of alcohol drinking, *ALDH2* and *ADH1B* genotypes on gastric cancer risk in the combination of HEPACC-2 and HEPACC-3

	HEPACC-2 + HEPACC-3				
	Ca/Co	Model 1 ^a		Model 2 ^b	
		OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value
Alcohol drinking status					
Never	470/688	Reference		Reference	
Light	315/580	0.83 (0.68–1.00)	0.049	0.87 (0.70–1.08)	0.217
Moderate	335/491	1.01 (0.83–1.23)	0.892	0.98 (0.78–1.22)	0.841
Heavy	244/276	1.33 (1.07–1.67)	0.011	1.19 (0.92–1.54)	0.178
Unknown	11/15				
		<i>P</i> for trend=0.017		<i>P</i> for trend=0.223	
<i>ALDH2</i>					
Glu/Glu	588/1018	Reference		Reference	
Glu/Lys	659/867	1.30 (1.12–1.50)	<0.001	1.46 (1.22–1.74)	<0.001
Lys/Lys	127/165	1.31 (1.01–1.68)	0.039	1.51 (1.09–2.10)	0.014
Lys+ (Glu/Lys + Lys/Lys)	786/1032	1.30 (1.13–1.49)	<0.001	1.47 (1.23–1.75)	<0.001
<i>ADH1B</i>					
His/His	836/1264	Reference		Reference	
His/Arg	477/698	1.03 (0.89–1.19)	0.690	0.98 (0.83–1.15)	0.812
Arg/Arg	61/88	1.07 (0.75–1.51)	0.722	1.07 (0.72–1.59)	0.730
Arg+ (His/Arg + Arg/Arg)	538/786	1.03 (0.90–1.19)	0.647	0.99 (0.84–1.16)	0.890
<i>ALDH2</i> and <i>ADH1B</i>					
Glu/Glu and His/His	368/627	Reference		Reference	
Glu/Glu and Arg+	220/391	0.96 (0.77–1.18)	0.688	0.90 (0.71–1.14)	0.364
Lys+ and His/His	468/637	1.23 (1.03–1.46)	0.022	1.38 (1.11–1.71)	0.004
Lys+ and Arg+	318/395	1.36 (1.11–1.66)	0.003	1.44 (1.14–1.82)	0.002

One case in HEPACC-2 was excluded because genotype was not defined

OR odds ratio, CI confidence interval, Ca case, Co control

^aCrude ORs (age- and sex-matched ORs) were calculated by a conditional logistic regression model

^bORs were calculated by a conditional logistic regression model adjusted for age, alcohol drinking status, pack-year of smoking, fruit/vegetable intake, family history, *H. pylori* infection and gastric atrophy

Table 3 Impact of alcohol drinking on gastric cancer risk according to *ALDH2/ADH1B* genotypes in the combination of HERPACC-2 and HERPACC-3

HERPACC-2 + HERPACC-3												
		<i>ALDH2</i> Glu/Glu		<i>ALDH2</i> Lys+		<i>ADH1B</i> His/His		<i>ADH1B</i> Arg+		<i>P</i> _{interaction}		
		Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	
Overall ^a												
Alcohol drinking												
Never	96/169	Reference		374/519	1.21 (0.86–1.71)	0.024	Reference	280/452	Reference	190/236	1.23 (0.93–1.63)	0.173
Light	155/298	0.97 (0.66–1.44)		160/282	1.04 (0.70–1.55)		0.98 (0.75–1.28)	203/366	0.98 (0.75–1.28)	112/214	0.88 (0.64–1.20)	
Moderate	165/311	0.94 (0.63–1.39)		170/180	1.58 (1.04–2.41)		1.16 (0.88–1.54)	214/282	1.16 (0.88–1.54)	121/209	0.93 (0.68–1.27)	
Heavy	164/230	1.09 (0.72–1.65)		79/46	3.57 (2.04–6.27)		1.30 (0.94–1.82)	132/155	1.30 (0.94–1.82)	111/121	1.26 (0.89–1.78)	
		<i>P</i> for trend = 0.221										
<i>H. pylori</i> infection (+) ^b												
Alcohol drinking												
Never	70/84	Reference		286/253	1.47 (1.01–2.14)	0.103	Reference	211/221	Reference	145/116	1.27 (0.93–1.74)	0.147
Light	124/155	1.07 (0.70–1.63)		115/146	1.08 (0.70–1.67)		0.89 (0.66–1.20)	155/185	0.89 (0.66–1.20)	84/116	0.79 (0.56–1.13)	
Moderate	132/183	1.03 (0.67–1.58)		141/90	2.22 (1.40–3.51)		1.21 (0.89–1.64)	173/158	1.21 (0.89–1.64)	100/115	0.98 (0.70–1.39)	
Heavy	133/132	1.28 (0.82–2.01)		59/21	3.59 (1.92–6.74)		1.30 (0.90–1.87)	106/81	1.30 (0.90–1.87)	86/72	1.19 (0.81–1.76)	
		<i>P</i> for trend = 0.381										
<i>H. pylori</i> infection (-) ^b												
Alcohol drinking												
Never	26/85	Reference		88/266	0.91 (0.51–1.63)	0.094	Reference	69/231	Reference	45/120	1.43 (0.88–2.33)	0.572
Light	31/143	0.75 (0.38–1.48)		45/136	1.05 (0.54–2.05)		1.06 (0.66–1.70)	48/181	1.06 (0.66–1.70)	28/98	1.18 (0.67–2.07)	
Moderate	33/128	0.73 (0.36–1.46)		29/90	0.90 (0.43–1.87)		1.15 (0.69–1.92)	41/124	1.15 (0.69–1.92)	21/94	0.75 (0.41–1.37)	
Heavy	31/98	0.73 (0.34–1.53)		20/25	2.13 (0.88–5.12)		0.98 (0.53–1.82)	26/74	0.98 (0.53–1.82)	25/49	1.57 (0.83–2.98)	
		<i>P</i> for trend = 0.488										

OR odds ratio, CI confidence interval, Ca case, Co control

^aORs were calculated by a conditional logistic regression model adjusted for age, pack-year of smoking, fruit/vegetable intake, family history, *H. pylori* infection and gastric atrophy

^bORs were calculated by an unconditional logistic regression model adjusted for age, sex, pack-year of smoking, fruit/vegetable intake, family history and gastric atrophy

of heavy drinking among *ALDH2* Lys allele carriers was observed in both HERPACC-2 and HERPACC-3 (Online Resource 4).

Table 4 shows the mediation effects between *ALDH2/ADH1B* polymorphisms and alcohol drinking on gastric cancer risk with or without consideration of *ALDH2/ADH1B* and drinking interaction. The mediation analysis indicated a significant direct effect of the

Table 4 Mediation effects of the *ALDH2* Lys alleles (vs Glu/Glu) and *ADH1B* Arg alleles (vs His/His) on gastric cancer risk in combination of HERPACC-2 and HERPACC-3

	HERPACC-2 + HERPACC-3			
	Model 1 ^a		Model 2 ^b	
	OR (95% CI)	P value	OR (95% CI)	P value
Model without consideration of <i>ALDH2</i> –drinking interaction				
Direct effect	1.57 (1.33–1.84)	<0.001	1.55 (1.31–1.84)	<0.001
Indirect effect	0.85 (0.80–0.91)	<0.001	0.89 (0.83–0.95)	0.001
Total effect	1.34 (1.15–1.55)	<0.001	1.38 (1.18–1.61)	<0.001
Model with consideration of <i>ALDH2</i> –drinking interaction				
Direct effect	1.66 (1.38–2.00)	<0.001	1.67 (1.38–2.03)	<0.001
Indirect effect	0.82 (0.75–0.89)	<0.001	0.84 (0.76–0.92)	<0.001
Total effect	1.36 (1.16–1.58)	<0.001	1.40 (1.19–1.64)	<0.001
	$P_{\text{interaction}}=0.114$		$P_{\text{interaction}}=0.042$	
Model without consideration of <i>ADH1B</i> –drinking interaction				
Direct effect	1.02 (0.88–1.18)	0.798	1.01 (0.86–1.18)	0.941
Indirect effect	1.02 (1.00–1.03)	0.022	1.01 (1.00–1.02)	0.218
Total effect	1.04 (0.89–1.20)	0.645	1.01 (0.87–1.19)	0.866
Model with consideration of <i>ADH1B</i> –drinking interaction				
Direct effect	1.02 (0.88–1.19)	0.788	1.01 (0.86–1.19)	0.876
Indirect effect	1.01 (0.99–1.02)	0.526	1.00 (0.98–1.02)	0.843
Total effect	1.03 (0.88–1.19)	0.733	1.01 (0.86–1.18)	0.892
	$P_{\text{interaction}}=0.105$		$P_{\text{interaction}}=0.148$	

One hundred and four in HERPACC-2 and one hundred forty-seven subjects in HERPACC-3 were excluded because of missing data of covariates

OR odds ratio, CI confidence interval

^aMediation variable, drinking status (category); Covariates (HERPACC version, sex, age)

^bMediation variable, drinking status (category); Covariates (HERPACC version, sex, age, pack-year of smoking, fruit/vegetable intake, family history, *H. pylori* infection and gastric atrophy)

ALDH2 Lys alleles on gastric cancer risk in each model. Furthermore, the interaction between *ALDH2* and alcohol drinking on gastric cancer was statistically significant, as the above conventional multivariate analyses showed ($P_{\text{interaction}}=0.042$). *ALDH2* Lys allele carriers showed a 67% increased risk of gastric cancer when *ALDH2*–alcohol drinking interaction was considered (multivariate-adjusted OR 1.67; 95% CI 1.38–2.03). In contrast, the indirect effect mediated by alcohol drinking was protective against gastric cancer with a risk reduction of 16% (multivariate-adjusted OR 0.84; 95% CI 0.76–0.92). The direct and indirect effects of *ADH1B* polymorphism were not significant, contrary to those of *ALDH2* polymorphism. Even when a regular quantity of drinks consumed per day (g/day, continuous variable) was set as a mediation variable, the mediation effects did not change significantly (data not shown). The results for HERPACC-2 and HERPACC-3 are shown in Online Resource 5 separately. The significant direct and indirect effects of *ALDH2* Lys alleles were consistently observed in both studies.

Discussion

Here, we consistently observed a significant association between *ALDH2* polymorphism (rs671) and gastric cancer risk in both this replication study (HERPACC-3) as well as in our derivation study (HERPACC-2). We also replicated our previously reported significant interaction between *ALDH2* polymorphism and alcohol drinking regarding gastric cancer risk [20]. Moreover, our mediation analysis indicated that the direct effect of the *ALDH2* Lys alleles was associated with an increase in gastric cancer risk independent of a change in alcohol drinking behaviors, whereas the indirect effect mediated by alcohol drinking was protective against gastric cancer. No evidence of a significant association of *ADH1B* polymorphism (rs1229984) with gastric cancer was observed.

The significant association between *ALDH2* polymorphism and gastric cancer risk identified here was consistent with most earlier studies [17–23]. The interaction between *ALDH2* and alcohol drinking on gastric cancer was also consistent with some previous studies [19–21], albeit that only a few studies have examined the interaction [18–21, 23]. A Korean case–control study with 445 cases and 370 controls showed that *ALDH2* Glu/Lys genotype had a significantly increased risk of gastric cancer associated with alcohol drinking ($P_{\text{interaction}}=0.048$) [19]. Similarly, a nested case–control study with 457 cases and 457 controls in Japan showed that *ALDH2* Lys allele carriers with alcohol consumption of ≥ 150 g/week had an approximately twofold higher risk than *ALDH2* Glu/Glu carriers with alcohol consumption of < 150 g/week, indicating a

marginal gene–environment interaction ($P_{\text{interaction}} = 0.08$) [21]. However, some of the previous case–control studies which indicated a significant association between *ALDH2* and gastric cancer failed to observe a significant interaction [18, 23]. Possible explanations for this discrepancy could be methodological differences in the categorization of alcohol consumption, sample size, adjusted confounders, and minor allele frequency among populations.

To explore the mechanism by which *ALDH2* polymorphism caused an increased risk of gastric cancer associated with alcohol drinking, we conducted a mediation analysis. The total effect of *ALDH2* polymorphism was thereby clearly decomposed into direct and indirect effects on gastric cancer. The observed significant *ALDH2*–alcohol drinking interaction and the direct effect of *ALDH2* Lys alleles on gastric cancer risk suggest that the direct effect may vary by alcohol drinking status; that is, the direct effect may operate more strongly for drinkers than never drinkers. This finding may be explained by the reduced enzyme activity in metabolizing alcohol-derived acetaldehyde among *ALDH2* Lys allele carriers. Specifically, *ALDH2* Lys allele carriers may be exposed to increased acetaldehyde after alcohol consumption. In separate studies, salivary acetaldehyde concentration among *ALDH2* Glu/Lys carriers after alcohol consumption was 2–3 times higher than that among *ALDH2* Glu/Glu carriers [35, 36], while the acetaldehyde concentration in gastric juice among *ALDH2* Glu/Lys carriers after intragastric alcohol infusion was 5.6-fold higher than that among *ALDH2* Glu/Glu carriers [37]. Furthermore, an experimental study showed that acetaldehyde-derived DNA adducts, which lead to carcinogenesis [38], in the gastric mucosa were higher among *ALDH2*-knockout or *ALDH2*-heterozygote mice treated with ethanol (*ALDH2* $-/-$ or $+/-$) than in *ALDH2*-active mice treated with ethanol (*ALDH2* $+/+$) [39]. These findings appear to consistently support the increased local alcohol-related acetaldehyde exposure and carcinogenic effect on the stomach in *ALDH2*-deficient populations.

The indirect protective effect mediated through alcohol drinking may indicate that *ALDH2* Lys allele carriers consume a lower amount of alcohol, which is in turn associated with a lower risk of gastric cancer. This protective effect may attenuate the direct effects of *ALDH2* Lys allele on increased risk of gastric cancer, which may be partly responsible for the inconsistent interaction between *ALDH2* and alcohol drinking in previous studies. If a conventional multivariate analysis does not appropriately adjust for alcohol drinking behavior, the protective indirect effect may prevent observation of the true effect of *ALDH2* on gastric cancer risk. Our findings might, therefore, highlight the importance of conducting mediation analyses as well as conventional multivariate analyses to explore the role of *ALDH2* polymorphism in the development of alcohol-related cancer.

The major strength of this study is its large sample size from two case–control studies, including detailed information on alcohol drinking. Several limitations should also be noted. First, information on alcohol drinking and smoking may be affected by recall bias. However, lifestyle information was collected before the first medical examination for outpatients, and any recall bias might, therefore, be relatively small. Second, unmeasured covariates may have confounded the conclusion of the mediation analysis. In mediation analysis, the following assumptions are required to control potential bias: (1) no unmeasured exposure–outcome confounding; (2) no unmeasured mediator–outcome confounding; (3) no unmeasured exposure–mediator confounding; and (4) no unmeasured mediator–outcome confounders affected by the exposure [30]. Our analysis likely upheld assumptions (1) and (3), because exposure was a genetic polymorphism in a single ethnic group [40]. Considering the specific effect of *ALDH2* polymorphism on alcohol drinking, it may be plausible to consider that assumption (4) was also upheld. However, it remains unclear whether assumption (2) was upheld, and we cannot deny the possibility that socioeconomic status, for example, might have confounded the association between alcohol drinking and gastric cancer, which would break this assumption. Nevertheless, we did control several important potential confounders, such as smoking, *H. pylori* infection and gastric atrophy, and therefore believe that the four assumptions may hold, to some extent at least.

In conclusion, we replicated the interaction between *ALDH2* and alcohol consumption in gastric cancer risk. The observed direct effect of *ALDH2* polymorphism may support the involvement of acetaldehyde-derived from alcohol drinking in the development of gastric cancer. The indirect effect may emphasize the importance of alcohol reduction in the prevention of gastric cancer.

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Compliance with ethical standards

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Informed consent Informed consent to be included in the study, or the equivalent, was obtained from all patients.

Conflict of interest The authors declare that they have no conflict of interest.

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